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# NUTRITION REVIEWS

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*August 1946*


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# NUTRITION REVIEWS

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## THE EXPANDING OPPORTUNITY OF THE SCIENCE OF NUTRITION

At the turn of the century a chemist who specialized in foods and nutrition could (if given time enough) analyze foods with as close an approximation to 100 per cent as is usual in the analysis of other natural materials; but he could not accomplish satisfactory nutrition with pure mixtures of the substances his analysis revealed. Yet the function of food is to nourish—so obviously some means to deeper insight was needed.

The dilemma was attacked along three main lines: physical chemistry was increasingly drawn upon both for viewpoints and implementation; structural organic chemistry was brought to bear upon the more complete differentiation and identification of substances present only in small amounts; and feeding experiments with animals of rapid growth and short natural life cycle were brought into more extensive and also more intensive use in studies of nutritive values and nutritional requirements.

The rewards of this broadening of research methods are truly epoch-making. Conspicuous as were the rapid series of vitamin discoveries—each the key to a previously baffling disease—that is only one side of the coin. And the other, while less dramatic, is no less worthy of our best attention.

For, it is of the greatest importance, both in principle and in practice, that the newer knowledge of nutrition is not only corrective but also constructive. Gradually and quietly, with no fanfare of discovery by any one person at any one time, recent experimental research has established the nutritional improvability of the norm. When a "new vitamin" has been discovered and qualitatively identified and even when the quantity needed for normal nutrition has been ascertained, our science of today no longer stops at that point, as if there were nothing more to be learned in that line, but presses on to the more ambitious and constructive problem: How much yields best results, not only in immediately measurable status of body, or rate of growth of the young, but throughout the whole life history and even through successive generations?

Thus today's science of nutrition is custodian of the potentiality not only to cure and prevent the diseases of malnutrition but also to build higher health and efficiency in many who are already passably healthy and efficient.

It is doubtful if most of the experimental workers in nutrition fully appreciate the far-reaching significance of this development in their science, because for nearly a century until the most recent years both chemistry and biology have handicapped themselves in this field by a too rigid acceptance of the dogma of the fixity (fixité) of the body's internal environment. Laboratory researchers should perhaps ask themselves whether this handicap of a too dogmatic limiting assumption tends to make them less far-seeing in their view of the significance of nutrition than are their most nutritionally-minded contemporaries in the professions of medicine and public health, and in the profession of economics and the world of affairs.

Nutrition Reviews has existed largely for the sake of rendering a continuous service to the mutual understanding of nutrition and medicine, and now it grows more apparent each month that there is also a corresponding opportunity evinced in the newer nutrition-consciousness of the world of affairs. News of the progress

of the United Nations Food and Agriculture Organization has already been reported in these pages. Concrete evidence of the thinking of leaders also appears in such individual contributions as that published under the title: "Redirecting world agricultural production and trade toward better nutrition" (F. F. Elliott, *J. Farm Econ.* **26,** *10 (1944)*).

The intellectual and political "climate" of today brings to the experimental science of nutrition a more far-reaching opportunity than hitherto could be realized or even anticipated. This opportunity—to serve human progress through a more nutritionally guided use of the world's food (and food-production resources) by the world's people—can be met by fuller use of the same general concepts and methods by which the great advances of recent years have been gained. The liberating concept of the flexibility and improvability of body processes and nutritional life histories, should be implemented by the most accurate and comprehensive use, not only of purchasable physical equipment, but also of the scientifically developed laboratory colony of experimental animals built by the researcher for the particular problem he undertakes. The building of this living instrument-of-precision is an extremely important feature of such research as is called for by the new opportunity here considered.

The well controlled laboratory colony of properly chosen species, from which experimental animals in sufficiently large numbers for conclusive statistical analysis of results and of like hereditary and nutritional backgrounds can be drawn at precisely known ages, grouped in strict parallel, and fed diets to be compared throughout entire lifetimes and successive generations, constitutes a quantitative research instrument of greater precision and significance than has hitherto been available.

With such a precision colony ably manned by people trained to the highest standards of accuracy through the rigorous disciplines of education in the exact sciences, the nutrition researcher of today and tomorrow can show much more clearly and objectively than was possible hitherto, just how different foods compare in nutritive values and just how improvements of food supply affect the nutritional status and thus the efficiency and the life history of the individual, or of families from generation to generation.

Such comprehensive feeding experiments have shown, among other things, that with some (we know not how many) nutrients there are unexpected wide margins between the merely (or minimal) adequate and the optimal level of intake. That is, by stepwise increments of intake in series of parallel experiments, one finds a level which supports growth and health with passable success in reproduction and lactation. A higher level of intake may then make no apparent difference in experiments as ordinarily conducted, but in the long run of more comprehensive and more searchingly quantitative investigations the complete life histories and records of experimental families through successive generations may show that the further increments of intake have brought further benefit to the long-run record of nutritional well-being as shown in the average of sufficient numbers of full-life and successive-generation tests. The fact that wide margins of beneficial liberality of intake have been found in this way in experimental studies of calcium, vitamin A, and riboflavin—and in other ways in studies of ascorbic acid—does not justify any assumption regarding other nutrients; for the four just mentioned were chosen for such long-time investigations not at random but because antecedent researches gave reasons for regarding them as promising. Of other nutrients, the beneficial margins may be much less. Each

deserves comprehensive and rigorous investigation entirely on its own merits.

Such investigations are exacting and time consuming but their findings are fundamental to our fuller understanding of nutritional life histories, and to the best guidance of health and of the use of food production resources.

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## PRESENT KNOWLEDGE OF THE MINERALS IN NUTRITION (PART II)

*Iron:* The need for iron in the human body varies greatly at different ages and under varying circumstances. During periods of increased demand for hemoglobin formation, such as growth, pregnancy, and blood loss, a diet deficient in iron produces an iron deficiency, resulting in a hypochromic, microcytic anemia. In the healthy adult male or in healthy women after the menopause, there is a very low, if not negligible iron requirement. Gastrointestinal disturbances, such as diarrhea, achlorhydria, or intestinal disease, may interfere with absorption sufficiently to prevent iron from entering the body, though it is present in the diet in adequate amounts. Wartime diets in Great Britain produced an increase in the anemia of iron deficiency in women and children (*Nutrition Reviews* **1,** *85* (*1943*)). Supplementing these diets with iron produced good hemoglobin responses (*Ibid.* **2,** *215* (*1944*); *Ibid.* **3,** *96* (*1945*)). Attention has also been drawn to the considerable iron loss by regular blood donors (*Ibid.* **1,** *293* (*1943*)).

The chief functions of iron in the body are the transport of oxygen to the tissues by the iron-containing pigment, hemoglobin, in the circulating blood, the buffering power of blood and muscle globins, and the oxidation in the tissues of metabolites through the medium of iron-containing compounds such as cytochromes.

The metabolism of iron in the human body is not well understood, despite the many interesting studies which have been made in the past few years. An important fact is that iron is not excreted in appreciable amounts by either the kidney or the intestinal tract. The human body is able to absorb ferrous iron more readily than ferric, although the reverse may be true in some experimental animals (*Nutrition Reviews* **1,** *154* (*1943*); **2,** *45* (*1944*); **3,** *110* (*1945*); **4,** *109* (*1946*)). The absorption of both ferrous and especially ferric iron is interfered with in man by phytic acid (*Ibid,* **1,** *269* (*1943*); **2,** *45* (*1944*)); it has been postulated that the phytic acid content of whole wheat flour is responsible for a lower absorption of iron from whole wheat flour than from white flour (*Ibid.* **1,** *59* (*1942*)).

Iron has been found to produce a typical hemoglobin response in those patients with vitamin B-complex deficiencies who had a hypochromic anemia (*Nutrition Reviews* **1,** *217* (*1943*)). This response could not be augmented by concurrent administration of the deficient vitamins. In no case did pyridoxine administration alone stimulate hemoglobin regeneration. The microcytic anemia of pyridoxine deficiency in the dog and the pig is characterized by elevated plasma iron levels, hemosiderosis in the liver, spleen, and bone marrow indicating that iron absorption was not interrupted (*Ibid.* **3,** *29* (*1945*)).

The Recommended Dietary Allowance for iron is 15 mg. per day for adolescent children and pregnant and lactating women, and 12 mg. per day for other adults. These are amounts which can be obtained readily

from any diet which is varied and adequate in other respects.

*Iodine:* The value of iodine in the prevention of endemic goiter has been widely recognized. The distribution of iodine varies with the local geologic conditions of the soil and water; the iodine of foods depends upon that of the soils and waters in the districts where they were grown. When the distribution of available iodine is low, the incidence of endemic goiter is high. Within the colloid of the alveoli comprising the thyroid gland, iodine is stored in the amino acids, diiodotyrosine and thyroxine. The iodine concentration in the normal thyroid is maintained at about 40 mg. per 100 g.; when this concentration drops below 10 mg. per 100 g., hyperplasia begins and may result in goiter (D. Marine and C. H. Lenhart, *Arch. Int. Med.* **3,** *66* (*1909*)). The only known physiologic importance of iodine is its occurrence in thyroxine, the secretion of the thyroid which maintains control of the energy metabolism of the body through some unknown pathway.

Human blood normally contains a fairly constant concentration of iodine, one fourth of which appears to be in combination with the circulating thyroxine (R. A. Davison and G. M. Curtis, *Proc. Soc. Exp. Biol. Med.* **41,** *637* (*1939*); R. A. Davison, R. W. Zollinger, and G. M. Curtis, *J. Lab. Clin. Med.* **27,** *643* (*1942*)). Studies with radioactive iodine have indicated that iodine is transported quickly to the thyroid by the circulating blood where it is converted rapidly into organic iodine compounds which may be returned to the circulation within a few hours (I. Perlman, I. L. Chaikoff, and M. E. Morton, *J. Biol. Chem.* **139,** *433, 449* (*1941*)).

The human requirement for iodine has been calculated in different ways, one of which is the total balance study. V. V. Cole and G. M. Curtis (*J. Nutrition* **10,** *493* (*1935*)) found that normal individuals under basal conditions of bed rest on a hospital diet under controlled conditions each required an average of 67 micrograms of iodine daily or approximately 1 microgram per kilogram of body weight. The Recommended Dietary Allowances suggested values of "0.002 to 0.004 mg. daily for each kilogram of body weight, or a total of 0.15 to 0.30 mg. daily for the adult." These values allow 1 to 3 micrograms of iodine daily for each kilogram of body weight, in addition to the basal requirement of 1 microgram per kilogram, to supply the needs occasioned by ordinary body functions and also some for reserves. The use of iodized salt regularly in cooking and on the table will provide somewhat more than the amounts recommended. The use of iodized salt during adolescence and pregnancy is sound preventive medicine.

*Copper:* Nutritional anemia develops in infants and in experimental animals when they are maintained exclusively on a whole milk diet. Prior to the observations of E. B. Hart, H. Steenbock, J. Waddell, and C. A. Elvehjem (*J. Biol. Chem.* **77,** *797* (*1928*)),iron was believed to be the only element deficient in a whole milk diet. However in the young rat which was made severely anemic through maintenance on a milk diet exclusively, no hemoglobin regeneration was found to occur unless a copper salt was fed in addition to a pure inorganic iron salt. Copper is not a constituent of the hemoglobin molecule and although it is an essential element in the nutrition of animals, its functions are not understood. Under certain circumstances of iron deficiency in anemic dogs, there is a more efficient utilization of iron for hemoglobin formation when copper is supplied above levels which are adequate when there is ample iron (*Nutrition Reviews* **1,** *11* (*1942*)). A reduction in cytochrome oxidase activity has been observed in the bone marrow of copper deficient rats (*Ibid.* **3,** *157* (*1945*)). In infections of various kinds, with fever, in experimental hyperthermia, serum copper levels decrease, then increase, while serum

iron shows a prolonged decrease (F. van Damme and J. Vandenbroucke, *Compt. rend. Soc. de biol.* **139,** *81* (*1945*)). A maximum serum copper of 318 micrograms per 100 ml. was observed, while the normal level was stated to be 145 micrograms. In the marine gastropods and arthropods, copper is a constituent of hemocyanin, a protein similar to hemoglobin which acts in the transport of oxygen (A. C. Redfield, T. Coolidge, and A. L. Hurd, *J. Biol. Chem.* **69,** *475* (*1926*)).

Copper deficiency has been observed in cattle which have grazed on pastures demonstrably low in copper. An anemia develops that cannot be cured by iron salts but the administration of copper produces a definite improvement (H. W. Bennetts and F. E. Chapman, *Australian Vet. J.* **13,** *138* (*1937*); abstracted in *Nutrition Abstr. Rev.* **7,** *794* (*1937–8*); S. Jamieson and F. C. Russell, *Nature* **157,** *22* (*1946*)). The human requirement for copper has been studied in several metabolic balance experiments. Since the body does not excrete copper readily, it has been difficult to make any accurate evaluation of the normal requirement. A daily allowance of 2.0 to 2.5 mg. of copper has been suggested for young women, which is an amount that can be readily obtained from average diets. (R. M. Leverton and E. S. Binkley, *J. Nutrition* **27,** *43* (*1944*)).

*Cobalt:* Cobalt was shown to be an essential nutrient in animal nutrition when three different groups of investigators found almost simultaneously that minute amounts of cobalt would relieve the progressive emaciation and anemia occurring naturally in the cattle and sheep of Western and Southern Australia (E. W. Lines, *Comm. Australian J. Council Sci. Ind. Res.* **8,** *117* (*1935*); H. R. Marston, *Ibid.* **8,** *111* (*1935*); and E. J. Underwood and J. F. Filmer, *Australian Vet. J.* **11,** *84* (*1935*); see "Handbook of Nutrition," *pp. 164–165, American Medical Association, Chicago* (*1943*)). The anemia produced in rats on a whole milk diet does not respond better when cobalt is given in addition to iron and copper (E. J. Underwood and C. A. Elvehjem. *J. Biol. Chem.* **124,** *419* (*1938*)). When anemia is produced in dogs by a whole milk diet or by bleeding, the administration of cobalt in addition to copper and iron sometimes aids blood regeneration (D. V. Frost, C. A. Elvehjem, and E. B. Hart, *J. Nutrition* **21,** *93* (*1941*)).

In a study of the toxicity of various metals (K. Waltner and K. Waltner, *Klin. Wchnschr.* **8,** *313* (*1929*)) it was found that when cobalt was added to the diet of rats there was a marked increase in the number of red blood cells (polycythemia). No cobalt was detected in tissues from pigs fed a milk, iron, copper, and manganese diet, but definite quantities were found in practically all the tissues from pigs fed a similar diet plus cobalt. The greatest amount was found in the liver, pancreas, and spleen (F. J. Stare and C. A. Elvehjem, *J. Biol. Chem.* **99,** *473* (*1933*)). If the rat requires cobalt it is in extremely small amounts only, less than 0.03 microgram per day, which is less than 0.7 microgram of cobalt per 1000 calories (A. E. H. Houk, A. W. Thomas, and H. C. Sherman, *J. Nutrition* **31,** *609* (*1946*)).

The human requirement for cobalt is unknown. Some favorable results with cobalt in the treatment of anemia in children have been reported (K. Kato, *J. Pediatrics* **11,** *385* (*1937*)). These results have not been confirmed and in adults negative results have been reported (C. R. Baxter, *Brit. Med. J.* **1,** *534* (*1939*) and E. Cronin, *Ibid.* **1,** *643* (*1939*)).

*Manganese:* Manganese deficiency has been produced in rats (E. R. Orent and E. V. McCollum, *J. Biol. Chem.* **92,** *651* (*1931*)) and in mice (A. R. Kemmerer, C. A. Elvehjem, and E. B. Hart, *Ibid.* **92,** *623* (*1931*)). Manganese deficiency was later shown to be one of the causes of perosis in growing chicks (*Nutrition Reviews* **2,** *50* (*1944*)). A low hatchability of eggs

laid by hens deficient in manganese has been described; the embryos which did develop had shortened leg and wing bones and distorted beaks (M. Lyons and W. M. Insko, *Poultry Sci.* **16,** *365* (*1937*)). A variety of deficiency signs have been observed in rats fed manganese deficient diets. Considerable controversy has arisen over the effect of manganese deficiency upon the rate of growth, estrus cycle, and reproductive ability in the rat. Probably many of the differences in deficiency signs have been due to differing degrees of acuteness of the deficiency as produced by diets of varying manganese contents (*Nutrition Reviews* **1,** *394* (*1943*)).

Little is known of the functions of manganese in the body. Manganese deficient chicks have a lower blood and bone phosphatase activity than normal birds; this change occurs before the development of perotic signs (A. C. Wiese and co-workers, *J. Biol. Chem.* **127,** *411* (*1939*)). *In vitro* experiments have shown that manganese can activate not only blood and bone phosphatases but also those in yeast, intestine, and liver. The phosphatases are also activated by other divalent ions, magnesium, cobalt, and iron but not as effectively in most cases as by manganese. Arginase, the enzyme which is involved in the elaboration of urea, is also activated by the manganese ion (S. Edlbacher and H. Pinösch, *Z. f. physiol. Chem.* **250,** *241* (*1937*)). Numerous other enzymes have been reported to be activated by this mineral.

There is no evidence of the occurrence of manganese deficiency in man. On the basis of balance studies, G. J. Everson and A. L. Daniels (*J. Nutrition* **8,** *497* (*1934*)) suggested that the diet of preschool children should contain between 0.20 and 0.30 mg. of manganese per kilogram of body weight. R. A. Kehoe, J. Cholak, and R. V. Story (*Ibid.* **20,** *85* (*1940*)) reported that the daily human diet contained approximately 4 mg. of manganese, which would seem to be in excess of the requirement.

*Zinc:* Zinc deficiency was produced in rats maintained on a diet containing only 1.6 parts per million of zinc (W. R. Todd, C. A. Elvehjem, and E. B. Hart, *Am. J. Physiol.* **107,** *146* (*1934*)). The growth of the rats deficient in zinc was much slower than the controls and a definite alopecia was produced over various parts of the body. Little is known of the physiologic role of zinc. D. A. Scott and A. M. Fisher (*Biochem. J.* **29,** *1048* (*1935*)) have shown that zinc is associated physiologically with insulin. Carbonic anhydrase is a zinc-protein complex (D. Keilin and T. Mann, *Nature* **144,** *442* (*1939*)). The enzyme uricase also may be a zinc compound (C. G. Holmberg, *Biochem. J.* **33,** *1901* (*1939*)). However no disorder in carbohydrate metabolism, no significant decrease in carbonic anhydrase activity, and no decrease in the concentration of uricase has been observed in zinc deficient rats.

At present little is known of the requirement of man for zinc. The data from balance studies indicate that there is some retention of zinc (F. I. Scoular, *J. Nutrition* **17,** *103* (*1939*); A. Stern, M. Nalder, and I. G. Macy, *Ibid.* (*Suppl.*) **21,** *8* (*1941*)). Scoular has recommended 0.3 mg. of zinc daily per kilogram of body weight. Since the zinc content of foods approximates that of iron, this amount is procured easily.

*Fluorine:* Fluorine became of interest as a public health problem when F. S. McKay, in collaboration with G. V. Black showed that mottled enamel was caused by some agent carried in communal water supplies (*Dental Cosmos* **58,** *477* (*1916*)). In 1931 three independent groups of investigators reported that fluoride was responsible for mottling (H. V. Churchill, *Ind. Eng. Chem.* **23,** *996* (*1931*); M. C. Smith, E. M. Lantz, and H. V. Smith, *Science* **74,** *244* (*1931*); H. Velu, *Compt. rend. Soc. de biol.* **108,** *750* (*1931*)). Mild mottling of the enamel occurs in only 0.6 to 2.0 per cent of the children in communities where the fluorine content of the water is between 0.6 and 1.2

p.p.m.; there is an increase in the severity and an increase in the percentage incidence of mottling when the the fluorine content is greater (*Nutrition Reviews* **1,** *182* (*1943*)). Toxic effects of fluorine on the skeletal system have been described (*Ibid.* **3,** *242* (*1945*); **4,** *145* (*1946*)). Bones and teeth always contain some fluorine but there is a tremendous increase in fluorine content when there is a high fluorine ingestion. The removal of fluorine from the skeletal system after ingestion of fluorine is decreased is relatively prolonged (*Ibid.* **1,** *110* (*1943*)).

In regions where the fluorine content of drinking water is 1 p.p.m. or greater, there is a lower incidence of dental caries in children residing in those communities during the periods of tooth development (*Ibid.* **1,** *182* (*1943*); **3,** *115* (*1945*)). When fluorine is applied topically to the teeth, a substantial reduction in the incidence of tooth decay has been reported frequently (*Ibid.* **2,** *149* (*1944*); **4,** *145* (*1946*)). However two such investigations yielded negative results (F. A. Arnold, H. T. Dean, and D. E. Singleton, *J. Dental Research* **23,** *155* (*1944*); B. R. East and co-workers, *Ibid.* **24,** *267* (*1945*)). The fluorine content of carious teeth has been reported to be lower than that in noncarious teeth (W. D. Armstrong and P. J. Brekhus, *Ibid.* **17,** *393* (*1938*)). The studies at Newburgh, New York, where sodium fluoride is being added to the drinking water supply in sufficient quantity to bring its fluorine content to about 1 p.p.m. will add much to our present knowledge of the relation of fluorine ingestion to incidence of dental caries (*Nutrition Reviews* **3,** *115* (*1945*)).

Fluorine is a poison in some enzyme systems, specifically inhibiting the production of phosphopyruvate from phosphoglycerate. *In vitro* experiments indicate an inhibition of esterases (A. S. Loevenhart and G. Peirce, *J. Biol. Chem.* **2,** *397* (*1906–7*)) and of phosphatases (S. Ochoa, *Ibid.* **138,** *751* (*1941*)). Since the various investigations on the relation of fluorine to dental caries have been reported, there has been some discussion and a little research on the possibility that fluorine may be an essential mineral nutrient. As yet studies with low fluorine diets have not provided any evidence that fluorine is essential for the growth of experimental animals (G. R. Sharpless and E. V. McCollum, *J. Nutrition* **6,** *163* (*1933*); R. J. Evans and P. H. Phillips, *Ibid.* **18,** *353* (*1939*)).

*Boron:* Boron has been shown to be essential for the growth of plants, and has also been shown to be present in small amounts in animal tissues. No reduction in the growth of rats has been observed when they were maintained on diets which supplied as low as 0.8 microgram of boron per day (E. Orent-Keiles, *Proc. Soc. Exp. Biol. Med.* **44,** *199* (*1940*); E. Hove, C. A. Elvehjem, and E. B. Hart, *Am. J. Physiol.* **127,** *689* (*1939*)).

*Aluminum:* Aluminum is widely distributed in plant and animal tissues and the human dietary contains a little over 10 mg. per day. It is unknown whether aluminum is an essential nutrient. Rats that have been fed a diet which supplied as little as 1 microgram of aluminum daily for a period of six weeks did not differ from the control rats (E. Hove, C. A. Elvehjem, and E. B. Hart, *Am. J. Physiol.* **123,** *640* (*1938*)).

*Vanadium:* Since none of the ordinary materials used to make purified rations for experimental animals and since none of the animal tissues examined contains vanadium, it is believed that vanadium is not an essential nutrient (*Nutrition Reviews* **1,** *8* (*1942*)).

There are many elements which occur in very small amounts in plants and animals which have not been investigated. The study of the essential nature of these materials constitutes a challenging field for the fundamental investigator. The probability that the deficiency of any one or more of these may occur in man seems rather remote at present because of their wide distribution in nature and the relatively minute requirement.

## PYRIDOXAL PHOSPHATE AS COENZYME OF TRANSAMINASE

Pyridoxine is known to be concerned (a) with the metabolism of tryptophane, by an unknown mechanism, and (b) with the enzymatic decarboxylation of amino acids, the derivative pyridoxal phosphate acting as a coenzyme (codecarboxylase) (*Nutrition Reviews* **3,** *72, 343 (1945)*). Synthetic codecarboxylase has been prepared by phosphorylation of pyridoxal, and recently purified as the barium salt (I. C. Gunsalus, W. W. Umbreit, W. D. Bellamy, and C. E. Foust, *J. Biol. Chem.* **161,** *743 (1945)*). Pyridoxal phosphate contains 1 atom of phosphorus per mole of pyridoxal. Standard acid hydrolysis liberates phosphate, a phenolic type group, and causes a characteristic spectral change, all indicative of a simple splitting to pyridoxal, and all proceeding parallel to loss of coenzyme function. The exact chemical structure of pyridoxal phosphate remains to be proved.

Bacterial carboxylases involving pyridoxal phosphate are recognized for tyrosine, lysine, arginine, ornithine, and glutamic acid, but the role of such decarboxylations in animal tissue is not clear. It is of interest to note, however, that the decarboxylation of 3,4-dihydroxy-phenylalanine ("dopa") also requires pyridoxal phosphate (D. E. Green, L. F. Leloir, and V. Nocito, *J. Biol. Chem.* **161,** *559 (1945)*), and dopa carboxylase may be isolated from an animal source, for example pig kidneys.

It now appears that pyridoxal phosphate also functions as a coenzyme for transaminases, the enzymes which transfer the amino group of glutamic acid to keto acids to produce new amino acids. These transaminases occur in animal tissues as well as bacterial cells, and are undoubtedly of importance in protein metabolism. Background for this new function of pyridoxine was the finding of F. Schlenk and E. E. Snell (*J. Biol. Chem.* **157,** *425 (1945)*) that tissues of pyridoxine deficient rats had low transaminase activities, and that this activity of the tissue could be increased by addition (*in vitro*) of pyridoxal and adenosine triphosphate, a combination known to parallel the effects of pyridoxal phosphate. E. E. Snell (*J. Am. Chem. Soc.* **67,** *194 (1945)*) also demonstrated the interconversion of pyridoxal and pyridoxamine by heating with the appropriate amino or keto acid, and suggested that a biologic function of these pyridoxine derivatives might be to transfer amino groups, the type of "carrier" function well recognized for other coenzymes. In possible support for this biologic role is the experiment of W. D. Bellamy, W. W. Umbreit, and I. C. Gunsalus (*J. Biol. Chem.* **160,** *461 (1945)*) in which the transformation of pyridoxamine to pyridoxal phosphate in the presence of pyruvate was demonstrated in Streptococcus faecalis.

On the other hand, considerable evidence had accumulated from the literature that no coenzyme was involved in transamination. The coenzyme postulated by M. G. Kritzmann (*Biokhimiya* **3,** *603 (1938)*; **4,** *667 (1939)*) could not be detected by P. P. Cohen (*J. Biol. Chem.* **136,** *565 (1940)*), and Green, Leloir, and Nocito (*loc. cit.*) attribute the "coenzyme" of aspartic aminopherase (transaminase) to traces of glutamic acid. P. P. Cohen and H. C. Lichstein (*J. Biol. Chem.* **159,** *367 (1945)*) could find no decrease in the transaminase activity of S. faecalis when grown on a pyridoxine deficient medium, although the decarboxylase activity was decreased. Finally, L. F. Leloir and D. E. Green (*Federation Proceedings* **4,** *96 (1945)*) found no evidence for a coenzyme during the preparation of purified transaminases, although when these enzymes were later specially tested for pyridoxal phosphate, positive results were obtained (Green, Leloir, and Nocito, *loc. cit.*).

The report of H. C. Lichstein, I. C. Gunsalus, and W. W. Umbreit (*J. Biol. Chem.* **161,** *311 (1945)*) leaves little doubt that pyridoxal phosphate is a coenzyme for

transaminases in S. lactis R. The transaminase enzymes were isolated in cell-free solutions from these bacteria, and methods of analysis for their assay were developed. Two principal methods were employed to demonstrate the coenzyme function of pyridoxal phosphate, (a) organisms grown in a medium deficient in pyridoxine were very low in transaminase activity, but preparations from these organisms were greatly stimulated by the addition of pyridoxal phosphate, and (b) the transaminase from organisms grown in pyridoxine mediums was partially split by aging and dialysis so that the protein fraction was reactivated by addition of pyridoxal phosphate. This "resolution" of the protein-coenzyme complex was not complete, but sufficient to demonstrate a significant reactivation by added pyridoxal phosphate.

In the study of Green, Leloir, and Nocito (*loc. cit.*) purified transaminases were tested for a heat-stable coenzyme by utilizing a test system of dopa carboxylase from which the coenzyme had been removed. This modified dopa carboxylase had been found to require codecarboxylase or pyridoxal phosphate for reactivation. The boiled transaminase preparations were found positive for decarboxylase, strongly indicating that transaminase contains a coenzyme, and that this is probably pyridoxal phosphate. The coenzymes of the transaminases showed similar pH stabilities to pyridoxal phosphate, and the coenzymes stimulated growth of L. casei in a pyridoxine free medium. It seemed evident that the coenzyme of transaminase is firmly bound to the protein, and even when the protein was denatured the coenzyme was associated with the protein. It was not possible to dissociate reversibly the protein-coenzyme complex. Prolonged dialysis against dilute ammonia caused inactivation of transaminase with parallel loss of its decarboxylase content, indicating at least that the decarboxylase is an essential part of transaminase.

Missing from the general picture is rigorous proof for the chemical structure of pyridoxal phosphate, and the exact mode of action of the coenzyme in its decarboxylation and transaminating functions. Progress has been most rapid however, considering that the special microbiologic activity of pyridoxal and pyridoxamine was announced by Snell and co-workers only two years ago. Particularly impressive in this field has been the skillful selection and culturing of bacteria to provide special systems for study, a demonstration of the need to employ a variety of technics and tissues in advancing the science of nutrition.

## NIACIN CONTENT OF FOODS

It is difficult to overestimate the importance of the development of adequate and reliable analytical data on food composition. Accurate tables of food composition provide information which permits the selection of foodstuffs which combine maximum nutritive value with economy of production. The wide practical application of the discovery of a new vitamin or other essential nutrient is usually dependent upon the rate at which information on the distribution of the new factor in foods becomes available. This new information in turn depends upon the development of practical analytical methods and the application of such methods to a large number of foods. Since the composition of each food varies greatly depending upon the conditions of growth, storage, and preparation, several years and the contributions of many investigators may be required before sufficient data are available about a particular nutrient to provide representative values adequate for general use in planning.

Nicotinic acid (niacin) has been recognized since 1937 as the pellagra-preventive factor and has been widely used in the treatment of pellagra. Important analytical data on the distribution of this dietary essential in foods has been reviewed previously (*Nutrition Reviews* **1,** *3* (*1942*)). A further contribution which confirms and extends the information in this field has been published by M. Swaminathan (*Indian J. Med. Res.* **32,** *39* (*1944*)). Analyses of 160 Indian foodstuffs are reported, and although some of them are uncommon to the American market, many are foods used the world over.

The analyses were accomplished with the cyanogen bromide method which has been shown to be reasonably reliable through comparison with independent microbiologic methods. The table summarizes the findings of Swaminathan in a manner which may be useful to the reader. The original publication should be consulted for more detailed information on particular foods. Most of the data given in the original publication appear to be the results of analyses of single specimens of a given food. There is therefore little information presented which allows one to judge the variation among different specimens of the same foodstuffs.

| FOOD | NIACIN | FOOD | NIACIN |
|---|---|---|---|
| | *mg. per 100 g.* | | *mg. per 100 g.* |
| Cereals | | | |
| Corn and oats | 0.6–1.3 | Leafy vegetables | 0.4–0.6 |
| Millet | 1.4–3.0 | Roots and tubers | 0.4–1.0 |
| Rice (polished) | 1.2 | Other vegetables | 0.2–0.6 |
| Rice (parboiled) | 4.0 | Fruits | 0.2–0.6 |
| Wheat | 5.0 | Milk | 0.1–0.2 |
| White flour | 1.0 | Eggs | 0.1 |
| Wheat germ | 7.0 | Muscle meat | 5–7 |
| Beans and peas | 1.3–2.5 | Liver | 14–16 |
| Nuts | 1.4–2.5 | Fish | 1.5–3.0 |
| Peanuts | 14 | Yeast (dried) | 20–60 |

It will be noted that of the foods tested, yeast, liver, meat, wheat germ, and peanuts are rich sources of niacin while wheat, millet, parboiled rice (i.e. steamed and dried before milling), legumes, nuts, and fish are good sources. Vegetables, fruits, milk, and eggs are poor sources of this particular nutrient.

The National Research Council recommends a daily dietary allowance of 12 to 20 mg. of niacin.

## BIOTIN DEFICIENCY AND SUSCEPTIBILITY TO INFECTION WITH SALMONELLA

Elsewhere in this journal reference to the relation of biotin deficiency to various types of infection has been made (*Nutrition Reviews* **3,** *345* (*1945*)). The susceptibility of chickens and ducks to malaria and of rats to Trypanosoma lewisi infection is known to be greatly increased in biotin deficient animals. I. J. Kligler, K. Guggenheim, and H. Herrnheiser (*J. Infect. Dis.* **78,** *60* (*1946*)) who have studied susceptibility to infection by Salmonella in vitamin A deficient rats, present the results of a study of the suscepti-

bility of rats and mice to this type of infection under conditions of biotin deficiency.

The basal experimental diet consisted of rice flour 81 per cent, olive oil 4 per cent, yeast 10 per cent, and salt mixture 5 per cent; 100 I.U. of vitamin A and 4 I.U. of vitamin D twice weekly were added. The deficient diet was prepared by adding 150 g. of raw egg white per 100 g. of ration. The controls received boiled instead of raw egg white. Typical biotin deficiency was reported. Growth of deficient animals ceased after seven to eight weeks.

Very young male weanling rats were used; none was pubescent at time of infection. This, perhaps, is significant in view of the suggested high biotin requirement of rapidly growing tissues (*Nutrition Reviews* **3,** *343* (*1945*)). Experimental infection was induced after fourteen, twenty-one, and twenty-eight days, respectively, on the raw egg white diet by feeding per os 0.1 ml. of a twenty-four hour broth culture of Salmonella typhimurium diluted 1:10 with milk. Animals were killed three days after exposure and cultures from liver, spleen, and mesenteric lymph glands were made. The data suggest that biotin deficient rats showed a markedly greater percentage of organ infection than the controls. The authors conclude that the decrease in resistance of biotin deficient rats might be due to increased permeability of the gut wall to the bacterium or to a lowering of cellular or humoral bactericidal ability.

The data suggest that infection was more likely to occur in deficient than in control animals. It is unfortunate that a somewhat more purified diet was not used, and that actual quantities of ration consumed were not indicated. Possible occurrence of multiple vitamin B-complex deficiencies on a restricted rice-yeast diet in the presence of excessive raw egg white must be considered in relation to the probable metabolic function of biotin (*Nutrition Reviews* **3,** *32* (*1945*)). More deficient than control animals appeared to be infected. It is difficult to attempt a simple comparison of the extent and rate of specific organ infectivity unless the percentage of infective "takes" in both control and deficient animals approximated 100 per cent. Where approximately 87 per cent of 130 deficient animals and 47 per cent of 130 control animals appeared to have "takes," some adjustments must be considered. H. C. A. Lassen ("Paratyphoid Infections in Avitaminotic Rats," *Levin and Munksgaard, Copenhagen* (*1931*)) points out that when using the organ culture method for demonstrations of variations in the resistance of experimental animals, several criteria must be considered: (1) The bacterial strain must consistently induce an infection, (2) the infection must be moderately severe but not usually fatal at its height, (3) the elimination of bacteria must take place in a uniform manner in terms of time and effectivity. His data indicate that with oral Salmonella infections of moderate intensity in the rat, the course of infection involves: (1) Peyer's patches and shortly thereafter mesenteric glands, (2) liver and spleen subsequently, (3) other lymphatics and organs eventually. Demonstrable persistent bacteremia apparently does not occur after oral infection. In many instances Lassen was unable to demonstrate progression of infection beyond the mesenteric glands in the three day postinfection period. Liver and spleen frequently did not become demonstrably involved until four plus days. In the experiment under consideration, it is interesting that at the third postinfection day, involvement of mesenteric glands was noted in 54 of the 61 infected controls (88.5 per cent), and 100 of the 113 infected deficient animals (88.5 per cent). The more marked discrepancy of apparent infection in liver and/or spleen may simply reflect a time phenomenon. No comment as to the ultimate end point and possible lethal outcome of infection is made It is questionable whether the data validate the conclusion:

"The decrease in resistance was reflected both in the incidence and in the manner of distribution of the bacteria."

In contrast to infection in the rat, data pertaining to the spontaneous epidemic of Salmonella typhimurium in mice deals with a lethal infection. Again the absolute incidence of infection appears to be significantly higher in the deficient group although 82 per cent of the deficient group and 93 per cent of the control group survived at least ten days. Virulent strains of Salmonella are presumably lethal to mice in six to twelve days (J. Ørskov and O. Moltke, *Z. f. Immunitatsforsch.* **59,** *357* (*1928*)). Incidence of oral Salmonella infection in mice appears to be independent of degree of absolute virulence of the infecting organism. The progression of the infection in rats, as described by Lassen, is similar to that described for mice by Ørskov. The data of Kligler *et al.* indicate that bacteria were recovered from 114 of 120 infected deficient mice (95 per cent) and 45 of 52 infected control mice (86.5 per cent). The difference would not appear to be significant. Possible temporal relationships complicate consideration of positive bacterial incidences in livers and spleens of deficient control animals.

While the work of Kligler *et al.* is stimulating when considered in association with other experiments previously discussed in these reviews, it is dubious whether "these experiments and observations provide an unequivocal answer to the question of whether biotin deficiency affects resistance to Salmonella infection." It is probable, however, that the influence of biotin deficiency on the resistance of the body to infection is a rather general one affecting the resistance to a wide variety of organisms.

## PALMAR ERYTHEMA AND PROTEIN DEFICIENCY

Palmar erythema, a redness of the palms of the hands, is an example of a fairly common but symptomless affection which has only recently attracted the attention of physicians. Though ancient Egyptian writings hint at the sign it was apparently first described and defined in modern times by A. J. Chalmers (*Lancet* **II,** *1514* (*1899*)) as an insignificant stigma occurring in Europeans living in a tropical region of West Africa. Dermatologists have been aware of it since the report of J. E. Lane (*Arch. Dermat. Syph.* **20,** *445* (*1929*)) on a type which affected several members of a family. E. N. Walsh and S. W. Becker (*Ibid.* **44,** *616* (*1941*)) noted the occurrence of palmar erythema in pregnant women. A comprehensive review of the medical papers on this subject was published by W. B. Bean (*Am. Heart J.* **25,** *463* (*1943*)) who had seen 11 cases in patients with hepatic or pulmonary disease. Because of the clinical association with hepatic disease and with pregnancy he advanced the hypothesis that the acquired form of this disorder was caused by an excess of circulating estrogens or related hormones. There was some inconclusive evidence to support this view (W. B. Bean, *Am. J. Med. Sci.* **204,** *251* (*1942*); R. C. Lofgren (*Arch. Dermat. Syph.* **46,** *502* (*1942*)) but not enough work had been done to establish this point.

A recent paper by R. M. Johnson and H. H. Hecht (*Am. J. Med. Sci.* **211,** *79* (*1946*)) gives further indication that palmar erythema is very common. These workers observed no fewer than 93 cases while examining 1183 patients in the Seymour Hospital, Eloise, Michigan during a period of thirteen months. Finding this sign in many different types of diseases the authors looked for some common denominator and were impressed by the frequent occurrence of protein deficiency as indicated by a low level of plasma albumin. In 73 of their 93 patients the mean level of albumin was 3.35 g. per 100 ml. and in the 40 controls the

mean level was 3.82 g., the difference being significant according to statistical criteria. Distribution curves for the plasma albumin concentration in the patients with palmar erythema as well as the controls were both partly outside the normal range, though the shape of the curve of the positive cases was the typical binomial variety while that of the controls was less regular. Since the exact cause is not known, the choice of a control group was necessarily somewhat arbitrary.

Evidence obtained by A. Segaloff (quoted by Johnson and Hecht, *loc. cit.*) did not support the suggestion that abnormally high levels of circulating estrogens produce palmar erythema. It should be remembered that the factor or factors responsible for the appearance of palmar erythema may have to be present for long periods, that there may be much variation in susceptibility, and that a study of patients at a time when the redness of the hands is already established may not reveal changes which were associated with its development. Though Johnson and Hecht are careful to state that they do not believe that hypoalbuminemia is a cause of palmar erythema it would appear from the data presented that their coexistence is more than coincidental. What the relationship may be is obscure. If the cause be a disturbance in estrogen metabolism resulting from hepatic dysfunction, the association of a low level of plasma albumin might well result from the same disorder in hepatic function. The suggestion that the finding of palmar erythema should make one suspicious of a protein deficiency should have further study which might elucidate the role of the liver in this situation, for it seems unlikely that palmar erythema would have been overlooked in such states as starvation edema when the hypoalbuminemia is much more severe. In the present state of our knowledge it appears that information on hepatic function, estrogen metabolism, and plasma protein concentration should be obtained in elucidating the pathogenesis of palmar erythema and in establishing its relationships.

## GINGIVITIS AND PLASMA ASCORBIC ACID LEVELS

The controversial question of the relationship of gingivitis to ascorbic acid nutrition has been reviewed (*Nutrition Reviews* **3,** *44 (1945)*). Equally controversial is the question of the significance for health of various plasma levels of ascorbic acid. Further data bearing on these points have now been reported by H. J. Leeson *et al.* (*Canad. Med. Assn. J.* **53,** *341 (1945)*). These investigators have studied over a three month period a series of 129 adult subjects in a mental hospital. The subjects were maintained on the regular institutional diet of which they had partaken for years and which was calculated to yield an average of not more than 20 to 25 mg. of ascorbic acid per day. Three groups of subjects were observed: a control group which received no supplementation to the regular diet; a group which was provided with a supplement of 100 mg. of synthetic ascorbic acid per day; and a second supplemented group which was provided with a daily allotment of grapefruit juice containing 58 mg. of ascorbic acid. The patients were carefully observed to make certain that these supplements were consumed. The supplementation was continued for a three month period. The subjects were examined at the beginning and end of the experimental period. The examination included macroscopic and biomicroscopic study of the gums and a detailed study of the skin and joints. Hemoglobin determinations and serum ascorbic acid estimations were made at the initiation of the study, after six weeks, and again at the end of nine weeks.

At the beginning of the experiment it

was noted that the oral hygiene of most subjects was poor. Gingivitis was recorded in some two thirds of the patients. An average plasma ascorbic acid level of 0.07 mg. per 100 ml. was found; 65 of the 129 subjects had no detectable plasma ascorbic acid. The hemoglobin levels were reasonably satisfactory—the mean value for men was 14.2 g. per 100 ml., for women, 13.2 g. It does not appear that clinical manifestations were encountered which could be considered diagnostic of scurvy.

Upon re-examination after three months of supplementation the average increase in plasma ascorbic acid levels for the control, fruit juice supplemented, and synthetic ascorbic acid supplemented groups was 0.22, 0.39, and 0.80 mg. for women and 0.06, 0.39, and 0.55 mg. for men, respectively. The increase in the mean level for the controls was attributed largely to a small group who apparently were given extra fruit. No significant changes were found in nutriture, weight, occurrence of hyperkeratosis, petechial hemorrhages, joint signs, gingivitis, or in hemoglobin levels as a result of the supplementations. "The only significant change detected was a rise in the plasma levels of ascorbic acid, but there was no indication that this had any beneficial effect."

The investigators conclude that an ascorbic acid intake of approximately 25 mg. per day is adequate for an adult in ordinary life. These findings are in close accord with those of W. P. Stamm, T. F. Macrae, and S. Yudkin (*Brit. Med. J.* **2,** *239 (1944); Nutrition Reviews* **3,** *44 (1945)*) involving 2961 members of the RAF. These men had had an ascorbic acid intake of from 15 to 27 mg. per day for a period of at least six months. Gingivitis was prevalent. Supplementation of well controlled groups with 100 to 200 mg. of ascorbic acid did not reduce the incidence of gingivitis. (See also W. J. Darby and D. F. Milam (*Am. J. Pub. Health* **35,** *1014 (1945)*).)

W. J. Linghorne and co-workers (*Canad. Med. Assn. J.* **54,** *106 (1946)*) have now reported an even more elaborate study of the relationship between gingivitis, ascorbic acid intake, and blood levels. This appears to be one of the most thorough studies of the subject on record. The authors conclude that unless amounts of ascorbic acid in excess of 25 mg. per day are ingested there is demonstrable handicap to the gingival tissues. They were only able to demonstrate such handicap after pretreating the mouths of the subjects of the study so as to remove existing infection around the teeth; and they attribute their own early failure and the failure of others to find any benefits from increased ascorbic acid intakes to the pre-existing infection.

A total of 350 Royal Canadian Air Force personnel were the subjects of the several studies. In the first experiment it was found that administration of ascorbic acid or niacin alone, or a combination of vitamin A, vitamin D, thiamine, riboflavin, niacin, and ascorbic acid for a period of five months resulted in no greater improvement in gingivitis than was observed in a control group. These subjects had received no special dental attention prior to institution of therapy.

In the final experiment, 150 subjects were given preliminary local treatment designed to remove all evidences of gingival inflammation. The treatment was completed by careful removal of calculus and polishing of the teeth. The striking effects of this local treatment in the clearing of gingivitis initially present are illustrated by the excellent color photographs accompanying the paper. The 120 persons who were able to complete the entire eight months of the experiment were grouped as follows: 33 on a mean dietary ascorbic acid intake of 7.9 mg.; 30 on an average daily ascorbic acid intake of 22.3 mg. (the same diet as the former group but supplemented with raw cabbage); 31 with an intake of 77.9 mg. of ascorbic acid daily (the same basal diet plus a tablet containing 70 mg. of ascorbic acid); and 26 receiving the regular station diet with an average

intake of 78.3 mg. Dietary control was maintained throughout and the serum levels of ascorbic acid served as a check on the intake of the factor. Photographic records of the gingivae were made periodically under carefully standardized conditions. A biopsy of a dental papilla was taken at the beginning and at the conclusion of the study. The dental examiners recorded bleeding tendency and tenderness employing a standardized technic which is described, and the degree of redness and swelling of the gums. At the end of the experimental period the incidence of these signs was increased in 45 per cent of the subjects receiving 7.9 mg. of ascorbic acid per day, in 29 per cent with 22.3 mg. per day, in 18 per cent with 77.9 mg. per day, and in 5 per cent with the regular station diet.

The differences in development of gingivitis between the higher intake groups and either of the two lower intake groups was statistically significant. With the small groups involved it was not possible to be certain that the differences between the two lower or between the two higher intake groups were real. In fact, the failure to observe differences in plasma levels of ascorbic acid of the groups with intakes of 7.9 and 22.3 mg. respectively may lead to some question that there developed a basic difference in the body stores of the vitamin in these two lower groups. It is, however, clear that body stores and serum levels were lower with both of these groups with restricted ascorbic acid intakes, and that there was a handicap to the gingival tissues.

Examination of the biopsy material revealed in no cases any specific changes which could be considered as characteristic of lack of ascorbic acid.

These studies demonstrate that provided existing gingivitis is eradicated the development of marginal gingivitis is more likely to occur on a diet containing 8 to 22 mg. of ascorbic acid per day than on a diet high in ascorbic acid. The data do not fix the minimal level at which this effect is apparent but indicate that it is in excess of 22 mg. per day. Although the results cannot be interpreted to indicate that all of the marginal gingivitis within the general population is prefaced upon ascorbic acid deficiency, they suggest that even moderate ascorbic acid deficiency can play a role in the development of such gingivitis. They also demonstrate that once such gingivitis is established, an improved ascorbic acid nutrition for a few months will not eradicate the condition.

## THE LIPIDS OF RAT TISSUES

H. H. Williams and his colleagues have reported extensive data on the lipid content of the rat's body as a whole, and of a number of different tissues, at various ages. H. H. Williams, H. Galbraith, M. Kaucher, and I. G. Macy (*J. Biol. Chem.* **161,** *463 (1945)*) described measurements of the total lipid content of the body, with special emphasis on differentiation of the total lipid into the storage or reserve fat and the essential or structural components. Measurements were made at different ages and the effect of dietary change was observed.

To observe the effect of age, animals from a single colony maintained on the Rockland stock diet were selected newborn, and at 15, 45, and 70 days of age. The whole body of each rat, less the intestinal contents, was ground in a food chopper, and dried under vacuum while still frozen. The dried solids were then extracted with hot ethanol and with ethyl ether. The combined extracts (about 3 parts of alcohol to 1 part of ether) were analyzed for choline, sphingomyelin, total and free cholesterol, galactose, and acetone soluble glycerol. Total phospholipid was determined by evaporating a portion of the combined extract in a stream of

nitrogen below 50°. The residue was extracted with petroleum ether and the petroleum solution was analyzed for total phospholipid. From the data so obtained the authors calculated total phospholipid, choline phospholipid, cephalin, lecithin, cholesterol esters, cerebrosides, and neutral fat by the method of M. Kaucher, H. Galbraith, V. Button, and H. H. Williams (*Arch. Biochem.* **3,** *203* (*1943*)).

The lipid content of newborn rats was found to be 21.26 per cent of the dry weight of the body, and this figure rose with age, being 26.68 per cent at 15 days, 38.23 per cent at 45 days, and 40.72 per cent at 70 days of age. The figures are the means for 28 newborn rats, 6-15 day old rats, 4-45 day old rats, and 2-70 day old rats. No data on the range of variation within the groups were given. The figures show that the total lipid content of the body almost doubled between birth and 10 weeks of age. This large increase was accounted for chiefly by increase in the neutral fat, or storage lipid, and did not represent actual "structural" changes occurring in the tissues. The neutral fat was 11.72 per cent of dry body weight at birth and rose to 35.50 per cent at 70 days. On the other hand the essential lipid fell from 9.54 per cent of the dry weight at birth to 5.22 per cent at 70 days, the phospholipid declining from 7.02 per cent to 3.63 per cent; the lecithin fell from 5.22 per cent to 1.35 per cent, while the sphingomyelin remained approximately constant at about 0.45 per cent and the cephalin increased somewhat from 1.37 per cent to 1.82 per cent. The cerebrosides, free cholesterol, and cholesterol esters all declined somewhat.

The authors also present the more significant picture given by the essential lipid expressed as the percentage of neutral fat free dry weight. This shows the essential lipid decreasing from 10.81 to 8.09 per cent between birth and 70 days of age. The greater part of this change had occurred in the first 15 days of life, when the essential lipid content had already fallen to 8.80 per cent. Expressed on the neutral fat free basis, the free cholesterol and the cholesterol esters, as well as the total phospholipids, all decreased in the first 70 days of life. Of the phospholipids, cephalin and sphingomyelin increased, but lecithin decreased markedly during this period. The cephalin accounted for one fifth of the phospholipids at birth but for one half at 70 days old. The total phospholipids fell from 74 to 69 per cent of the essential lipids, while the essential lipids fell from 45 to 13 per cent of total lipids.

The comparative effect of three different diets was also studied. The diets all contained casein, sucrose, salt mixture, Crisco, dextrin, and vitamin supplements. The Crisco and dextrin together supplied 77.2 per cent of the calories in each diet, but the proportions of the two were different, so that in one diet each furnished 38.6 per cent of the calories. A second diet, high fat, furnished 67.9 per cent of the calories as Crisco, and the third, low fat, supplied only 9.3 per cent of the calories as Crisco. The control rats, killed at weaning time when the experimental animals were started on one of the three diets enumerated, showed the lipid content of the rats at the beginning of the experiment. All experimental rats (killed after consuming 3000 calories of the experimental diet) showed much storage of fat, but as expected this was due to accumulation of neutral fat and the changes in the essential lipids could be demonstrated only by expressing the results as percentages of neutral fat free dry weight. The low fat diet produced the smallest, and the high fat diet the greatest, storage of neutral fat.

The changes in the essential lipid distribution in the bodies of the rats receiving a moderate or high fat diet were those expected only as a result of advancing age. The rats on the low fat diet however showed certain distinct changes; the content of cerebrosides was lower and the content of cholesterol esters higher than in the rats on the diets furnishing more than 10 per cent of calories as fat.

H. H. Williams and co-workers (*J. Biol. Chem.* **161,** *475 (1945)*) have also described measurements made on the different tissues of the rat at similar ages. To obtain these data rats from the same colony mentioned above were anesthetized with ether, exsanguinated from the aorta, and the heart, thymus, lungs, liver, kidneys, spleen, testes, brain, and muscle tissue from the legs and back were removed. These tissues were frozen and dried, and then extracted as described for the dried total body tissue. One hundred and sixteen rats at 15 days of age, 13 at 45 days of age, and 6 at 70 days of age, were used, although it is not clear whether each analytical figure reported is a mean for the separate analyses of the given tissue from each rat, or whether it represents a single analysis of the pooled tissues from all the rats of the given age group.

All of the tissues were found to increase in essential lipid content during growth from the fifteenth to the seventieth day; this increase is chiefly a reflection of increase in the phospholipid content, the major component of the essential lipids. The other components, cerebrosides and cholesterol, were found to vary independently with respect to each other and the total, during the same period of growth. The most basic change appeared to be an increase in the cephalin concentration. Specific changes were also observed for certain organs. These included an increase in cerebrosides in testes and skeletal muscle but a decrease in heart muscle, a decrease in free cholesterol and in sphingomyelin in both skeletal and heart muscle, and an increase in sphingomyelin in kidney, lung, and spleen. In the brain all essential lipid components except lecithin and cholesterol esters showed a marked increase during growth.

In discussing these data the authors stress the probability that these changes follow changing structural patterns in the several organs, and that the essential lipid components actually represent structural substances.

## SELENIUM ELIMINATION AND DISTRIBUTION IN EXPERIMENTAL EWES

Since seleniferous areas supply a portion of the meat consumed in the United States, the retention and elimination of selenium from the tissues of livestock might be important from the public health viewpoint. I. Rosenfeld and O. A. Beath (*J. Nutrition* **30,** *443 (1945)*) made a study of the effects of feeding and then discontinuing toxic doses of selenium upon the selenium content of the tissues and of the urine in yearling ewes. Five ewes were maintained on each of three diets which contained 20, 12, and 1.5 per cent of digestible protein, respectively. Each experimental ewe was given an oral administration of 10 mg. of selenium daily for twenty-one days; the daily dosage was then increased to 20 mg. until toxic signs appeared, and the selenium feeding was discontinued in 2 intoxicated ewes and increased to 30 mg. daily in the remaining 3 animals in each group. The selenium administration was stopped in order to study the rate of elimination from the tissues and the length of time required for the urine to become free of selenium.

The average daily excretion of selenium in the groups on the high, medium, and low protein diets during the first thirty-three days was 4.8, 5.5, and 3.5 mg., respectively. As the administration of selenium was continued beyond this point, there was a decreased ability to eliminate selenium, due to kidney injury. Toxic signs appeared in the ewes on the low protein diet after 360 mg. of selenium had been administered in gradual doses; toxic signs did not appear in the ewes on the medium and high protein diets until 860 mg. of selenium had been administered.

When selenium feeding was discontinued, the ewes on the medium and high protein

diets eliminated about twice as much selenium per day as those on the low protein diet. By the fiftieth day, the urines in all three groups were free of selenium or contained only traces. The tissues of the ewes which were on the medium and high protein diets and which received selenium for the entire experimental period, contained much higher amounts of selenium than those in the low protein group. The tissues of the 2 ewes in each group which received no selenium for the last sixty-one to sixty-four days of the experiment contained a great deal less selenium than the tissues of the ewes which received selenium daily throughout the experiment. In all groups the liver and kidney contained the highest levels while the brain and muscle stored the least. Fat was observed to be free of selenium in all the experimental animals regardless of the amount fed.

The ewes on the high and medium protein diets appeared to have a greater ability to store selenium and to excrete it than those on the low protein diet. In some way the higher protein intake had made it possible for the ewes to tolerate much greater amounts of selenium through these two mechanisms and possibly others. It is possible that the high protein intake had made available sufficient of the amino acids containing sulfur to increase the amount of selenium which could be stored by the replacement of sulfur and the formation of modified proteins with these amino acids.

These authors believe their data indicate that the meat from livestock slaughtered during a period of high selenium intake would contain sufficient selenium to be harmful in the human diet. However, if a period of sixty days or more had elapsed since the intake of selenium, the amounts contained in the meat would be insufficient to be injurious. Normally, human urine contains no selenium; in seleniferous regions, it may contain 100 to 200 micrograms per cent of selenium (M. I. Smith, K. W. Franke, and B. B. Westfall, *Pub. Health Reports* **51,** *1496 (1936)*). Undoubtedly part of the selenium thus eliminated must have come from meat produced in seleniferous areas.

## NUTRITIVE VALUE OF THE PROTEINS AND OIL IN TOBACCO SEED

L. B. Mendel and H. B. Vickery (*Conn. Agr. Exp. Sta. Bull.* **339** (*Part I*), *609 (1932)*) investigated the nutritive properties of the seed from Connecticut shade grown tobacco. A diet which consisted of whole ground tobacco seed, supplemented only with cod liver oil and inorganic salts, supported fair growth in albino rats and mice. They believed that vitamins B, E, and G were present in whole tobacco seed in sufficient amounts for growth, well-being, and reproduction but that the amounts of vitamins A and D were inadequate.

An oil meal was prepared from tobacco seed by K. E. Rapp, J. T. Skinner, and J. S. McHargue (*J. Nutrition* **31,** *261 (1946)*) by subjecting finely ground seed to a pressure of 2500 pounds per square inch in a Carver press until no more oil was expressed. This oil meal had a composition of: crude protein (N $\times$ 5.34) 25.8 per cent, ether extract 21.5 per cent, crude fiber 24.5 per cent, and moisture 5.0 per cent. Groups of rats were fed diets in which this tobacco seed oil meal was used as the sole source of protein, at levels sufficiently high to supply 15 or 18 per cent crude protein. The average increases in weight during the first six weeks were 23 and 32 g. respectively. When the oil meal was autoclaved and then fed at a sufficient level to supply 18 per cent protein, even poorer growth was observed. In efforts to determine the supplementary value of proteins from various sources, casein, lactalbumin, gelatin, and wheat gluten were individually incorporated at a 5 per cent level into a

ration which already contained sufficient tobacco seed oil meal to supply 10 per cent protein; likewise, the proteins of skim milk, rolled oats, and corn meal were tested for supplementary effect by the incorporation of sufficient of each crude material to supply 5 per cent protein in the ration containing 10 per cent tobacco seed proteins. The ration containing casein, lactalbumin, or skim milk produced better growth than the rations which contained only tobacco seed proteins. Gelatin and rolled oats were less satisfactory protein supplements, while wheat gluten and corn meal gave no supplementary effect. Since gelatin is deficient in cystine, 0.5 per cent cystine was added to the tobacco seed oil meal ration which had been supplemented with gelatin; no supplementary effect was produced by this means. Since both wheat gluten and the proteins in corn meal have a low lysine content, it was suspected that tobacco seed proteins might be deficient in lysine. When the ration containing sufficient tobacco seed oil meal to supply 15 per cent crude protein was supplemented with 0.5 per cent lysine hydrochloride, good growth resulted.

The coefficients of digestibility and the relative biologic values of the proteins in tobacco seed oil meal and in milk were determined. When present in sufficient amount to supply 10 per cent protein, the average true digestibility of the proteins in tobacco seed oil meal was 78.0 while that of the proteins in skim milk powder was 96.6. The biologic value of the oil meal proteins was 51.4, while that of the skim milk powder proteins was 78.5. When the oil meal was supplemented with lysine, the true digestibility was not altered but the biologic value was increased to 73.8.

The same authors have studied the nutritive value of the oil in tobacco seed (*J. Nutrition* **31,** *273 (1946)*). Tobacco seed oil was prepared by extraction from seeds with a low-boiling petroleum fraction, evaporation of the solvent and partial bleaching with fuller's earth, or by pressure-expressing from the finely ground seed. At first the solvent extraction oil was used; then since the expressed oil was found to have the same physical and chemical constants as the bleached extracted oil, the latter was used in subsequent experiments. Digestibility coefficient determinations were made for tobacco seed oil, cottonseed oil, and butterfat at 5, 15, and 30 per cent levels of fat intake. All rations were kept in the refrigerator. The average coefficient of digestibility for tobacco seed oil was 97.9 for the three levels, as compared with 99.1 and 98.2 for cottonseed oil and butterfat, respectively. The relative values of tobacco seed oil, cottonseed oil, and butterfat for growth promotion were determined at levels of 15 and 30 per cent of the diet. These rations were stored at room temperature. The average weekly gains of the rats with the 15 per cent tobacco seed oil ration were negligible, while the rats with the 30 per cent ration died in about twelve days. It was observed that the tobacco seed oil rations became spongy and had a pronounced odor. To determine the effect to which the nutritive value of the tobacco seed oil was affected by rancidity, a group of rats was fed the 30 per cent tobacco seed oil ration stored at room temperature, while another group was fed the same ration except that it was kept refrigerated. Excellent growth was observed in the rats fed the refrigerated ration, while those fed the ration kept at room temperature grew poorly. Supplementing the rancid ration with fat soluble vitamins, B-vitamins, or with both did not counteract the effect of the rancidity. The animals which survived the experiments with refrigerated and nonrefrigerated tobacco seed oil rations were changed to stock ration in mated pairs to determine if the reproductive ability had been impaired. The animals were not sexually mature at the end of the experimental period but when maturity was reached, all animals were found to be fertile. No trials were made to determine if the refrigerated tobacco seed oil

rations could support reproduction and lactation.

When refrigerated rations containing 30 per cent tobacco seed oil and butterfat, respectively, were compared by the paired feeding method, the rate of growth produced by the two rations did not differ significantly. When the rats were allowed to consume the rations ad libitum, the rats receiving the butterfat ration consumed more of it and grew somewhat more rapidly than those on the tobacco seed oil ration.

These studies form an interesting evaluation of the nutritive value of the oil meal and the oil from a seed which is not known to have been used as a food and which has never been produced in larger amounts than needed for starting the next year's crop. Studies with the rat have shown that the oil meal from tobacco seed contained proteins which were low in lysine but which had a good nutritive value when supplemented with adequate lysine hydrochloride. The oil from tobacco seed was found to have a high digestibility and to promote good growth providing the rations containing it were refrigerated. Thus for commercial use the oil meal would have to be supplemented with an adequate source of lysine and some means would have to be found to reduce the development of rancidity.

To obtain tobacco of higher quality and better uniformity, there has been an increasing practice among tobacco growers to harvest and cure individual leaves as they mature, instead of cutting the stalk and curing the entire plant. In fields where individual leaves are harvested, the stalks are allowed to stand in the field after all usable leaves have been harvested until a convenient time to turn the stalks under the soil. Therefore Rapp *et al.* suggested that it might be possible to produce and collect a considerable quantity of mature seed without affecting the quality of the leaves. These workers analyzed tobacco seed for nicotine and nornicotine but found neither of these substances. The extent to which tobacco seed products can be used as foods will depend probably on more extensive feeding trials with larger animals through the entire life cycle, as well as upon the seed yield per acre and upon the costs of harvesting and processing.

## GROWTH AND MAINTENANCE OF DOGS ON PURIFIED DIETS

The nutritional requirements of the dog have been studied extensively by a number of workers, and this subject has already been reviewed in this journal (*Nutrition Reviews* **2,** *298* (*1944*)). L. Michaud and C. A. Elvehjem reported that several hundred dogs had been reared successfully on purified diets containing sucrose, casein, cottonseed oil, and salts, supplemented with vitamins A and D and with thiamine, riboflavin, niacin, pyridoxine, pantothenic acid, and choline. It was felt therefore that the dog's nutritional requirements had been satisfactorily met.

This conclusion, however, did not seem to be in accord with the work of J. P. Lambooy and E. S. Nasset (*J. Nutrition* **26,** *293* (*1943*)) who reported that an additional factor or factors in yeast were required for optimum growth and appearance of fur and skin. Young dogs developed a dermatitis after seventy-five to one hundred and twenty-five days, and died in one hundred to one hundred and fifty days, when placed on a diet containing 35 per cent vitamin free casein, 41 per cent sucrose, 18 per cent hydrogenated cottonseed oil, 4 per cent bone ash, and 2 per cent salts, supplemented with thiamine, riboflavin, niacin, pantothenic acid, pyridoxine, choline, inositol, para-aminobenzoic acid, and vitamins A, D, E, and K. However, when yeast was fed at a level of 10 per cent in the diet (replacing an equivalent amount of casein) the dogs

remained healthy and grew rapidly. The dry yeast could be replaced by an alcohol soluble yeast or liver concentrate, or by kitchen scraps. Hemoglobin values ranged about 12 g. per cent. Although only 7 dogs were used throughout the entire study, the experimental values seemed clearcut.

Similar results were obtained by S. G. Smith (*Am. J. Physiol.* **142,** *476* (*1944*)), who also used a high fat, high protein diet, supplemented with 2 per cent cod liver oil and the same eight B-vitamins as were used by Lambooy and Nasset. Smith observed that the hemoglobin values in dogs receiving yeast averaged about 18 g. per cent compared with values ranging from 10 to 14 g. per cent among dogs on the purified diet.

None of the purified diets used by the three groups of investigators contained biotin. The value of biotin in canine nutrition has been recognized by S. G. Smith in a subsequent note (*Science* **100,** *389* (*1944*)) in which she reported that a progressive ascending paralysis developed in dogs on the purified diet, which could be cured either by the feeding of yeast or by subcutaneous administration of 100 micrograms of biotin per kilogram of body weight.

Another feature of the diets used by Lambooy and Nasset and by Smith was their very low choline content (0.2 and 5 mg. per kilogram per day respectively). This was recognized by the former group, and was also pointed out by W. R. Ruegamer, L. Michaud, C. A. Elvehjem, and E. B. Hart (*Am. J. Physiol.* **145,** *23* (*1945*)). Ruegamer and co-workers have reinvestigated the adequacy of purified diets, and their results are helpful in clarifying the situation.

Using a high fat, high protein diet it was found that the inclusion of 50 mg. of choline per kilogram per day permitted excellent growth of 2 dogs. The hair was reported to be lustrous and the lesions observed by the other workers failed to appear. The choline requirement is probably at least 50 mg. per kilogram per day, according to the work of J. M. McKibbin, S. Thayer, and F. J. Stare (*J. Lab. Clin. Med.* **29,** *1109* (*1944*)) and P. J. Fouts (*J. Nutrition* **25,** *217* (*1943*)), so that the diets used by Lambooy and Nasset and by Smith were probably seriously deficient in choline.

With a purified diet of lower fat content (sucrose 66 per cent, casein 19 per cent, cottonseed oil 11 per cent, salts 4 per cent) dogs were found to develop varying degrees of achromotrichia, as well as a slight anemia. Hemoglobin values ranged from 11 to 14 g. per cent. These values were then raised to 16 to 16.5 g. per cent by the administration of either 5 micrograms of biotin per kilogram or 3 liters of mineralized milk (estimated to contain 150 micrograms of biotin) per day, over a period of three and one-half months. The achromotrichia was apparently not improved, although inositol and para-aminobenzoic acid deficiencies were studied as possible causes. Similar observations regarding hair pigmentation were made by D. V. Frost and F. P. Dann (*J. Nutrition* **27,** *355* (*1944*)).

The Wisconsin group have discovered still another explanation for the failure of dogs to do well on the basal ration used by Smith (W. R. Ruegamer, C. A. Elvehjem, and E. B. Hart, *Proc. Soc. Exp. Biol. Med.* **61,** *234* (*1946*)). Apparently the diet contained very little potassium. Dogs placed on this diet by Ruegamer *et al.* ceased to grow and eventually became paralyzed. The paralysis and growth failure were observed whether or not the ration was supplemented with choline (50 mg. per kilogram per day) or biotin (7 micrograms per kilogram per day). The addition of potassium chloride to the diet promptly overcame the paralysis and induced a rapid growth response. Furthermore, a discoloration and erosion of the dental enamel, which was observed prior to the potassium therapy, was also benefited but not completely cured by the potassium supplementation. It remains to

explain the discrepancy between the failure of biotin to prevent paralysis in these experiments, and the positive results obtained with biotin by Smith.

On the basis of our present knowledge, it appears that satisfactory growth and fairly good hemoglobin formation in dogs can be realized with purified diets when the B-vitamin supplement includes only thiamine, riboflavin, niacin, pantothenic acid, pyridoxine, choline, and perhaps biotin. However, the best hemoglobin values and pigmentation seem to be obtained by the inclusion of natural supplements, such as yeast, into the diet.

In reviewing this series of studies, the reader is struck by the importance of critically evaluating experimental results. All known dietary constituents should be carefully tested and provided in ample amounts before the existence of new factors is reported. Even when new factors are found to exist, failure to separate their effects from those of known factors wherever possible, may lead to confusion and unnecessary duplication of effort.

## INFLUENCE OF DIET ON LIVER LIPID CONCENTRATION

Following a three week period of feeding on a low protein diet with no vitamin B supplement, the administration of a liver extract or of biotin was observed to produce fatty livers in one week unless inositol was also given as a supplement (G. Gavin and E. W. McHenry, *J. Biol. Chem.* **132,** *41 (1940)*; **134,** *683 (1940)*; **139,** *485 (1941)*; **141,** *619 (1941)*). Further investigation of this subject has been reported by P. Handler (*Ibid.* **162,** *77 (1946)*), in an attempt to determine whether biotin or any other factors present in the liver extract play specific parts in the regulation of the liver lipids or whether they merely increase the demand for lipotropic factors by stimulating the appetite and growth of rats previously stunted by an incomplete diet.

Male rats were grown to 80 g. weight and then depleted on a 10 per cent casein diet with no B-vitamins for three weeks. Ninety-five per cent of the rats survived this period and weighed 55 to 70 g. at the end of it. They were then given one of four diets, plus a daily injection furnishing the following amounts of vitamins (in micrograms); thiamine 25, riboflavin 30, calcium pantothenate 100, pyridoxine hydrochloride 20, nicotinamide 50, and para-aminobenzoic acid 100. The four diets were (a) a diet similar to the depletion diet, but containing 0.4 per cent cystine, (b) a high fat diet otherwise similar to the depletion diet, (c) the depletion diet with 1 per cent sulfasuxidine added, and (d) a 10 per cent fat diet containing also 12 per cent casein, 1 per cent sulfasuxidine, 0.4 per cent cystine, and 0.5 per cent cholesterol. Other supplements given to certain groups of the rats alone or in combination were choline (30 mg.), inositol (30 mg.), biotin (5 micrograms), "folic acid" (5 micrograms), alpha-tocopherol (200 micrograms), naphthoquinone acetate (20 micrograms), and 0.1 ml. refined corn oil as a source of unsaturated fatty acids.

With the low fat diet, plus the basal vitamin B supplement, fatty livers were obtained. These were prevented by choline, and to a lesser extent by inositol. The administration of liver extract caused fatty livers which were only partially prevented by choline, somewhat more by inositol, but prevented completely only by a combination of the two. Biotin also produced fatty livers prevented only by a combination of choline and inositol. The effect of the liver extract in producing fatty liver could be almost duplicated by a combination of "folic acid" and biotin. With the high fat diet, fatty livers were produced in the presence of either choline or inositol without

the addition of liver extract, biotin, or "folic acid," but were prevented by the combination of the two lipotropic factors. The effects of liver extract or of biotin were even more pronounced on the high fat than on the low fat diet.

The results mentioned above were obtained with a seven day feeding period after depletion; however, when the period was extended to twenty-four days, choline alone was sufficient to prevent the fatty livers. Experiments were made with rats depleted and also subsequently fed on diets containing sulfasuxidine to inhibit the synthesis of biotin, "folic acid," and inositol by intestinal microorganisms. The results were essentially similar to those already mentioned, except that biotin alone, or "folic acid" alone, exerted less effect in such rats. In contrast to rats not receiving sulfasuxidine, a requirement for inositol was still evident after a twenty-four day period of feeding choline, "folic acid," and biotin.

The production of fatty livers by the administration of biotin plus a basal supplement of B-vitamins, in the presence of adequate amounts of choline, was accompanied by considerable increase in food consumption and growth. This food consumption increase was intensified by the simultaneous feeding of "folic acid," although "folic acid" alone did not produce this effect. While biotin and "folic acid" increased the demand for lipotropic factors under the special conditions used, there was no evidence that they play a specific part in lipid metabolism. When liver extract or biotin and "folic acid" were included in the diet together with the basal vitamin B supplement after depletion, there was a much greater food consumption than when only those B-vitamins normally needed were given.

It was considered that under these circumstances the demand for the lipotropic factors (choline and inositol) would be increased. The demand for inositol slackened however as the appetite and growth stimulation caused by the "folic acid"-biotin combination fell off, when the rats had attained approximately the same size as would rats maintained on similar basal diets plus B-vitamins. At this point the intestinal flora might again be able to take over the task of supplying all the needed biotin, "folic acid," and inositol.

## EFFECTS OF INCREASED DIETARY INTAKES ON CHILDREN IN AN INSTITUTION

The growth curve of experimental animals has been an invaluable tool for the laboratory investigator in nutrition. Similar applications of growth measurements for man have not been so widely employed despite the development of useful standards (*Nutrition Reviews* **1**, *250* (*1943*)). A reason often given for this lack of application of height-weight data is the difficulty of interpretation. While some difficulties of interpretation are encountered, there is probably less difference of opinion as to the significance of weight data for the health of adults than there is regarding the meaning of most biochemical measures of vitamin content of body fluids and some of the proposed physical signs of deficiencies. For example, the characteristics of mortality among persons of abnormal weights are definite. These are epitomized by L. I. Dublin and A. J. Lotka ("Length of Life," *The Ronald Press Co., pp. 202–204, New York* (*1936*)) as follows: "Underweights succumb more readily to tuberculosis and the respiratory diseases than do persons of normal weight, but, on the other hand, show a lesser liability to diabetes and the degenerative diseases. In contrast, overweights show a superior resistance to tuberculosis, but experience an excessive mortality from diabetes and the degenerative diseases, the excess generally increasing with the

degree of overweight." It appears, therefore, that greater attention should be given to growth data and height-weight relationships.

Growth may be restricted by deficient intake of calories, protein, minerals, or vitamins, and by intercurrent infections or disease processes generally. Furthermore, there are seasonal fluctuations in growth rate (C. E. Palmer, *Pub. Health Reports* **48,** *211* (*1933*)), as well as favorable and unfavorable "growing" years (C. E. Palmer, *Ibid.* **48,** *993* (*1933*)). In studies of growth in man it is important to recognize the multiplicity of these factors which affect the process. Control of these factors is possible when employing experimental animals, but in man the investigations must meet practical considerations and, hence, they often fall short of critical tests of hypotheses. Failure to recognize limitations of the human studies has sometimes led to claims beyond the significance of observations. Nevertheless, valuable data can result from studies of the growth of children under various dietary conditions with and without supplementation. A noncritical bibliography has been compiled of experiments in which the effects of dietary supplementation have been investigated (Report by the Committee on Diagnosis and Pathology of Nutritional Deficiencies, National Research Council, *Arch. Int. Med.* **74,** *258* (*1944*)).

A study of the effect of dietary supplementation of children within an institution for a period of one year has been reported (L. J. Roberts, R. Blair, and M. Greider, *J. Pediatrics* **27,** *393*, *410* (*1945*); H. Oldham, L. J. Roberts, and M. Young, *Ibid.* **27,** *418* (*1945*)). This investigation differs from previous ones in that a variety of foods were added to bring the dietary pattern up to an "advised pattern" which included daily quantities of 1.5 to 2 pints of milk, 1 egg, 3 pats of butter, 1 serving of meat, 1 or more servings of vegetables other than potatoes, 1 or more servings of potatoes, 2 or more servings of fruit, and whole grain or enriched cereals. This dietary pattern met the recommended allowances of the Food and Nutrition Board, except for calories which some of the subjects voluntarily restricted to a level below the recommended allowances. The supplemented diet provided a 33 per cent increase in caloric intake, a 43 per cent increase in protein intake, and increases of from 73 per cent to 135 per cent for riboflavin, calcium, ascorbic acid, and thiamine. The vitamin A content of the diet was slightly decreased (minus 9 per cent) during supplementation.

The subjects lived in a boarding school and were girls from 6 to 10 years of age. Some of the children lived in the school, others came in for the day and ate two or three meals a day there for five or six days a week. The investigation continued over the period of a year, hence some of the original subjects were lost. The final analyses of growth data included 151 children who were separated into two groups, children who were present throughout ("throughout" group) and children present for but a portion of the year ("part-time" group). A comparison was made of the weight gains of the two groups before and after supplementation. The children present throughout the study gained a greater percentage of the expected weight than did those present for shorter periods or those who attended irregularly. The interpretation of this finding entirely in terms of dietary differences is made difficult by the probability that those children who attended irregularly may have done so because of illness. However, a convincingly greater weight gain occurred in both groups during the period of supplementation than during the preperiod. Thus, the "throughout" group gained 79 per cent of the expected weight during the preperiod and 142 per cent during supplementation. There was a less pronounced increase in the percentage of expected height gains during the period of supplementation—a gain of from 88 per cent of the expected for the "throughout" group

in the preperiod to 107 per cent during supplementation. It is important to note that the measurements during the preperiod were for only a two month interval while those during supplementation were for a year. The measurements of changes in height over a brief span of two months are less accurate than over a twelve month interval.

The authors observe that "It is a generally accepted theory that the upper limits of a child's growth in stature, at least, is set by heredity and that because of inadequate nutrition or other factors he may fail to attain it, but that he will not exceed his hereditary pattern. The better growth in height of these children in the proportion that they receive the more liberal diet would suggest that the original diet was susceptible of improvement." While one may agree with this interpretation of these data, the thesis must be regarded as unproved because of the absence of a true control group. As has already been noted, the part-time group does not provide adequate controls for such experiments. An adequate control would consist of a simultaneously observed unsupplemented group because it is recognized that there occur good and poor growth years for children. Thus, C. E. Palmer (*Pub. Health Reports* **48,** *993* (*1933*)) found that 7 year old boys gained a mean of 5.18 pounds in 1923 and 4.54 pounds in 1924. Later studies by this investigator (*Ibid.* **48,** *1277* (*1933*)) implied that poorer general economic conditions were not necessarily associated with a poor growth year. Hence, it becomes imperative to employ simultaneous controls in experiments designed to test the effect of dietary supplementation on the rate of growth of children.

Oldham, Roberts, and Young, (*loc. cit.*) also made laboratory examinations of blood levels of vitamin A, carotene, ascorbic acid, serum protein, hemoglobin, and thiamine, and urinary excretion studies of thiamine and riboflavin. These measures of nutriture were made while the children were receiving the institutional diet and again after supplementation had been instituted. The findings are of particular interest in view of the widespread use of these determinations in efforts to assess nutritional status. The results may be summarized as follows: The mean vitamin A content of the serum was the same before and after dietary alteration, a finding consistent with the fact that no change had been made in the vitamin A intake. The average plasma ascorbic acid level rose from 0.65 mg. per 100 ml. prior to supplementation to 0.83 mg. during supplementation. The average daily dietary intake had been increased from 38 to 81 mg. There was no significant change in the serum protein content after supplementation. Likewise, the increased iron intake during the period of supplementation was accompanied by no increase in hemoglobin concentration. The average iron intake prior to supplementation was 9.0 mg. for the 7 and 8 year old children and 10.7 mg. for the 10 and 12 year olds. The blood thiamine content increased from a mean of 5.5 micrograms per 100 ml. before supplementation on a daily intake of 543 micrograms, to 7.3 micrograms after supplementation which had increased the intake to 1.272 mg.

Simultaneously, four-hour urinary excretion tests following oral doses of 75 micrograms of thiamine per kilogram of body weight indicated increased returns after supplementation. Mean figures were not reported. Similar tests of riboflavin excretion did not reveal an increase in the amount of riboflavin recovered, a finding which was interpreted to indicate that the riboflavin content of the institutional diet was satisfactory. This intake ranged from 1.15 to 1.6 mg. per day for the several age groups.

This laboratory and dietary study indicates again that these commonly employed estimates of nutriture do in fact parallel dietary intake and are, therefore, useful in determining the level of intake of a group. They also give a better concept

of the quantitative relationship between these measures in the age group 6 to 10 years. The data do not define a lower limit of desirable intake or of laboratory assessments because the observed changes in the clinical criterion (growth) chosen cannot, in these studies, be attributed to one or another of the various dietary factors which were altered during the supplementation.

In the evaluation of experimental data on correlations between growth, size, level of dietary intake, and health, nutrition workers must clearly distinguish a number of related but distinct concepts. Thus, data on growth rates cannot be interpreted in terms of knowledge of relationships between health and adult stature (ultimate size). One should clearly distinguish between "increased" and "improved" growth rates. The two may not be synonymous. S. Brody (*Ann. Rev. Biochem.* **4,** *383* (*1935*)) has cautioned that "The nutritionists' ideal of rapid growth in the young seems incompatible with the desire for prolongation of life." Further, when increased weight status results from an increased consumption of almost all known dietary essentials one cannot define the factor responsible, but until proved otherwise it is logical to attribute the results to increased caloric intake unless it is demonstrated that a deficiency of other factors was limiting weight prior to supplementation. To further the public health field work in nutrition, additional investigations must be made, aimed at defining the relationships between (1) simple practical methods of judging nutritional status and health performance, and (2) the level of intake and the useful laboratory assessments of nutriture.

## ANEMIA IN A PIG WHICH RESPONDED TO PURIFIED LIVER EXTRACT

The production in an animal of anemia which responds to purified liver extract of the antipernicious anemia type has been the goal of many investigators for a number of years. The attainment of such a goal would make possible the assay of antipernicious anemia substances in animals, something which as yet can be done only in patients with pernicious anemia in relapse, and would also elucidate the pathogenesis of this disorder. This goal has, in particular, been the object of the investigations of Wintrobe and associates who have studied nutritional deficiencies and anemias in pigs for a number of years.

When these investigators fed young pigs crude casein together with the vitamins, thiamine, riboflavin, niacin, pyridoxine, choline, pantothenic acid, para-aminobenzoic acid, and inositol, excellent growth took place and no abnormalities were noted. G. E. Cartwright, M. M. Wintrobe, and S. Humphreys (*J. Lab. Clin. Med.* **31,** *423* (*1946*)) consequently tested the effect of a similar regimen modifying it only in that Borden's Labco vitamin free casein was given in place of the crude casein, and sulfasuxidine was fed to inhibit the growth of intestinal bacteria. Sulfasuxidine was added to the diet in an amount of 2 per cent. Since the diet was very expensive the vitamin free casein was given to one animal only.

After about sixty-five days, thinning of the hair over the rump and back was noted. The pig began to develop an untidy appearance. By about ninety days there was almost complete denudation over the flanks and rump, black crusts appeared about the eyelids, and there was definite impairment of growth. Unlike animals given a diet lacking in pyridoxine or in pantothenic acid (M. M. Wintrobe and coworkers, *J. Nutrition* **24,** *345* (*1942*)) the gait at all times was not remarkable.

On the one hundred and twentieth day of the experiment 1 mg. of biotin was given intramuscularly and this was continued

daily for seventeen days. No change occurred in the general appearance, coat, or growth of the animal, nor was the blood picture changed. On the one hundred and forty-first day of the experiment well marked anemia was present, the volume of packed red cells having fallen to 21 ml. per 100 ml. of blood. Unlike the anemia in pernicious anemia, however, the cells were essentially normal in size. As occurs in pernicious anemia the free protoporphyrin in the erythrocytes was in the lower range of normal for pigs. The plasma iron which had previously been elevated was approximately normal in amount while the serum copper was definitely elevated.

Purified liver extract assayed at 15 units of antipernicious anemia principle per cubic centimeter was given in ten daily doses of 1 ml. each. By the fifth day a definite reticulocyte response had occurred which reached a maximum of 9.4 per cent on the tenth day of therapy. There was an immediate rise in hemoglobin and volume of packed red cells and the plasma iron, after some fluctuation, rose to its previously high level. Serum copper fell slowly until it reached the normal range and there was a slight transient rise in the erythrocyte protoporphyrin during the reticulocytosis. Following the initial response there was a gradual increase in the hemoglobin and volume of packed red cells to the lower range of normal and a marked resumption of growth took place. Fifty days after the liver therapy the animal had gained 30 kg. in weight and a new, complete glossy coat of hair had appeared. The animal, except for moderate stunting of growth, looked normal.

A critical factor in the development of this anemia seemed to be the feeding of purified casein; the administration of sulfasuxidine to 4 animals fed a diet containing crude casein failed to produce the picture described.

The significance of this observation will have to await further studies. Since "folic acid" deficiency can be induced by feeding sulfasuxidine (W. H. Sebrell, *Harvey Lectures* **39,** *288 (1943–44)*) it might be assumed that the deficiency described in the pig was due to lack of one or more of the "folic acids." Yet, since it was relieved by administration of concentrated liver extract, a substance known to contain little Lactobacillus casei factor (G. W Clark, *Am. J. Med. Sci.* **209,** *520 (1945)*), another explanation must be found. In this connection one is reminded, however, of the observation that pernicious anemia itself can be relieved by the administration of L. casei factor (*Nutrition Reviews* **4,** *102 (1946)*), a fact which causes one to wonder about the "potential folic acid" activity of liver extract (*Ibid.* **4,** *15 (1946)*). Although the disorder described in the pig shows no relationship to pernicious anemia if one judges by the skin changes which developed and the normocytic instead of macrocytic anemia, the relief produced by concentrated liver extract and the recent studies linking the L. casei factor to pernicious anemia make it appear that some connection between the experimental disorder and the human disease may exist.

## EFFECT OF VARIOUS COCOAS ON CALCIUM UTILIZATION

Studies by W. S. Mueller and M. R. Cooney at Massachusetts State College on the relation of dietary cocoa to the retention of calcium and phosphorus have been reviewed previously (*Nutrition Reviews* **2,** *68 (1944)*). Even when rats on the cocoa supplemented diet received an 11 per cent higher caloric intake than the control rats, there was a 10.6 per cent slower average growth rate for the males and a 12.3 per cent slower rate for the females. These reductions could be attributed only to the

cocoa which was present in the diet. The data on calcium and phosphorus retentions were even more striking, with both male and female rats on the cocoa diet retaining approximately 15.0 per cent less of these minerals per 100 g. of body weight than the control rats. This decrease could not be explained by the amount of oxalic acid in cocoa.

H. H. Mitchell and T. S. Hamilton (*J. Nutrition* **31,** *377 (1946)*) continued this investigation by somewhat similar technics using three different kinds of cocoa: "the low-cost brand used by the Massachusetts investigators and two medium-cost products, an American process breakfast cocoa and a Dutch process cocoa." The oxalic acid content of the three cocoas was essentially the same. In these experiments cocoa was incorporated into the basal diet at the expense of sugar and all diets were equalized to compensate for the varying fat content of the cocoas. Again the amount of cocoa used was much above what is ever found in human dietaries. Additional amounts of sugar were given to the slower growing animals, those receiving the cocoa, so that the body weights would be equal to those of the controls. This additional amount of sugar in per cent of the basal diet consumed, averaged 18.9 for the low cost brand of cocoa, 6.3 for the American process breakfast cocoa, and 5.0 for the Dutch process cocoa. The rats on the cocoa diet received more calcium and phosphorus but stored considerably less in their carcasses than their litter mate controls which received cocoa free diets. The average depression in calcium retention was 27.7 per cent for the low cost cocoa, 14.8 and 18.4 for the American and Dutch process cocoas, respectively.

Some experiments of this type have been performed with human subjects by H. H. Mitchell and J. M. Smith (*J. Am. Med. Assn.* **129,** *871 (1945)*). The cocoa used was similar to the medium cost American process breakfast cocoa used in the rat experiments and was added to the diets in varying amounts ranging from 5.5 to 34.8 g. daily. The 5.5 g. level was selected since this amount approximates the average daily consumption in the United States and is the amount of cocoa contained in 400 ml. of chocolate milk containing 1.4 per cent cocoa. The results of these experiments were negative since no deleterious effects on the calcium metabolism were produced by these amounts of this particular brand of cocoa. These data may mean that cocoa in these amounts had no effect on the calcium utilization of human subjects or that the method of study was not sensitive enough.

Since a significant difference was observed between the reduction of calcium retention produced by a low cost cocoa and that produced by the two medium priced cocoas tested in rats, it would be of interest to test the effect of this low cost cocoa in human subjects for comparison with the negative result observed by Mitchell and Smith. Likewise it would be of interest to see if the low cost cocoa impaired growth and retention of calcium and phosphorus in the rat when used in the ration at a level comparable in body weight to what might be used in the daily diet of man.

## FATTY ACIDS IN MILK FAT GLYCERIDES

T. P. Hilditch in his book ("The Chemical Constitution of the Natural Fats," *Wiley, New York (1941)*) discussed the calculation of the distribution pattern of the fatty acids among the glycerides of natural fats. This can be obtained from the fatty acid composition if the percentage of saturated glycerides has also been determined, and

hydrogenation can be used at times to establish the location of the unsaturated constituents. As a result of his observations Hilditch states that "Any individual triglyceride molecule tends towards maximum heterogeneity in its composition. This... is in fact, the main principle which seems to be operative in the structure of the natural fats." He also remarked that in certain animal fats (particularly in milk fats) the fatty acids are even more widely distributed than in most vegetable fats.

On the other hand H. E. Longenecker and associates (*Chem. Rev.* **29,** *201* (*1941*); *Federation Proceedings* **1,** *122* (*1942*); *Oil and Soap* **21,** *73* (*1944*)) consider that the fatty acids are randomly distributed, requiring that all possible combinations be present according to the relative proportions of the component fatty acids. Suitable probability equations would then permit the calculation of the extent of the occurrence of each type of glyceride. In cow's milk, with a relatively high content of oleic and palmitic acids, one would therefore expect to find significant quantities of triolein and tripalmitin.

E. L. Jack, J. L. Henderson, and E. B. Hinshaw (*J. Biol. Chem.* **162,** *119* (*1946*)) have made a detailed analysis of the glycerides of milk fat and have compared the analytical results with expectations from these two hypotheses. Milk fat was dissolved in Skellysolve A, cooled to −7°, and the precipitate filtered off. Other solid fractions were obtained by filtration after standing at −13°, −23°, and −53° respectively, and the final filtrate at −53° formed the last fraction of the milk fat. The fatty acid composition of the glyceride fractions was determined by ester fractionation as described by E. L. Jack and J. L. Henderson (*J. Dairy Sci.* **28,** *65* (*1945*)). The saponification number of the various fractions were determined, together with the iodine numbers of all fractions except those of steam-volatile acids. The percentage of fully saturated glycerides in each fraction was determined and compared with a limiting minimum value of saturated glyceride percentage calculated from the analytical figures on the assumption that the unsaturation is distributed one fatty acid to a glyceride. It was also compared with a similar figure calculated on the basis of random distribution of the fatty acids among the triglycerides. The fatty acid composition of the fully saturated triglycerides in the various milk fat fractions was also determined.

The results appear to show that the distribution pattern of the unsaturated fatty acids tends more nearly to the pattern of widest distribution than to the pattern of random distribution. The "association ratio" (the ratio between the saturated and the unsaturated fatty acids present in the mixed glycerides) for the various fractions was found to be on the average 1.80. For the whole of the milk fat it was 1.76, thus supporting strongly the idea that milk fat has the same distribution pattern of saturated and unsaturated fatty acids as the various fractions. The further assumption therefore appears valid that the saturated fatty acids are also distributed as widely as possible, as are the unsaturated acids.

## NUTRITIONAL NEUROPATHY

Grossly abnormal neurologic symptoms in sick internees and prisoners of war have been described by C. A. Clarke and I. B. Sneddon (*Lancet* **I,** *734* (*1946*)). This group had been held by the Japanese from the fall of Hong Kong, in December, 1941, until September, 1945. Within three months of imprisonment, ankle swelling

appeared. This was followed by weakness and paresthesia of the limbs, difficulty in walking, and failure of vision and hearing. After one year skin lesions and stomatitis suggesting pellagra were noted. At this stage a distressing symptom, described as "electric" or "jumping" feet, was common. This was characterized by a burning sensation and extreme hyperesthesia of the soles of the feet. The skin symptoms and, to some extent, the "electric" feet responded to niacin which became available in the prison camps in 1943.

Optic atrophy and paralysis of the lower limbs were found in 74 of 200 medical and surgical cases removed to hospitals following the liberation of the camps. The most constant finding was impairment of visual acuity due to blind areas in the central visual field. The fundi showed partial optic atrophy. Deafness occurred and was usually bilateral. Characteristic signs of polyneuritis, except calf tenderness, were seen. Some cases showed such marked increases in deep reflexes, spasticity, and lack of muscular control that the spinal cord was believed to have been damaged. Blood changes were not significant and no definite relationship between the severity of symptoms and achlorhydria was evident. Plasma protein was generally normal and edema was not a prominent symptom at the time of liberation.

The day's ration consisted mainly of 0.5 to 0.75 pound of rice, usually polished, and 0.1 pound of dried fish. Inconstant additions were beans, peanut oil, and green vegetables (often chrysanthemum leaves). The caloric value during the four year period was maintained between 1600 and 1700. Protein decreased from 52 g. in 1942 to 33 g. in 1945. The intake of calcium, iron, vitamin A, and ascorbic acid averaged 0.27 g., 10 mg., 8000 I.U., and 70 mg., respectively. However, the thiamine intake ranged from only 0.27 to 0.50 mg. and the riboflavin from 0.17 to 0.65 mg. No estimate of the niacin intake was made.

Following release and hospitalization, these patients were given high calory diets and massive vitamin therapy, and those suffering from muscular incoordination were given re-educational exercises. The results at the end of two months were disappointing in that the neurologic signs, especially the partial or complete loss of vision, remained unaffected.

The authors refrain from ascribing the neurologic signs to simple vitamin deficiency. The fact that these appeared in almost epidemic proportions within a few months of captivity, reached a maximum, and then tended to remain stationary despite a decrease in the apparent nutritive value of the diet suggested to them that a toxic agent in rice was operative in the earlier months of captivity. That this is a possibility is well illustrated by the toxicity under certain conditions of peas of the genus Lathyrus. There is need of more information on the question of the factors which predispose the organism to nutritional disease. As H. B. Lewis (*Nutrition Reviews* **2,** *97 (1944)*) has stated, "The problems of lathyrism, favism, and related conditions, and diseases of nutrition associated with intoxicants should be investigated. It is not unlikely that other disturbances due to similar toxic factors exist and may be important, particularly in periods of food shortage or among peoples whose nutrition is at a minimal level." In this connection it is of interest that M. N. Rudra and K. P. Bhattacharya (*Lancet* **I,** *688 (1946)*) have reported that the serum phosphatase in 10 patients with lathyrism was greatly increased, the minimal value being over 200 per cent higher than normal values. The inorganic phosphate of the serum was also high. They suggest that circulating cocarboxylase may be destroyed in this condition so that the patient is functionally deficient in thiamine even though chemically saturated with the vitamin.

# NOTES

## Radioisotopes from the Manhattan Project

A detailed announcement on the availability and procurement of pile-produced radioisotopes from the Manhattan Project appears in the June 14 issue of *Science*.

Tables are included giving pertinent data on the characteristics and the quantities which may be made available of around 100 isotopes and isotopic mixtures. For practical reasons isotopes with a half-life less than 12 hours are not considered for distribution. Most of the isotopes are produced by fission or (n, $\gamma$) processes. Only four isotopes are produced by the (n, p) process with sufficient yield for distribution. Other processes are either not sufficiently productive or do not occur.

The article emphasizes that (1) present piles were not designed for tracer and therapeutic isotope production, (2) waste plutonium process solutions are not a feasible source for separated fission isotopes, (3) routine production methods and facilities are not yet developed for most isotopes, (4) isotopes which can now be made available are only experimental lots resulting from research and development proceedings, (5) technical problems involved in the irradiation and processing of essential materials has been and will continue to be responsible for the delay in making certain isotopes available by routine production.

Allocation and distribution will be effected on the basis of the general policies, as well as on recommendations regarding specific applications, made by well qualified advisory groups nominated for Manhattan District appointment by the National Academy of Sciences. Charges will be made for materials and services on the basis of "out-of-pocket" operational expenses to the government necessitated by the nonproject production and service program. Costs for construction or rental of major plant facilities and the expenses of research and development on isotope production will be assumed by the Project.

All correspondence concerning radioisotope procurement should be addressed to the Isotopes Branch, Research Division, Manhattan District, P. O. Box E, Oak Ridge, Tennessee. Reference to the original article for pertinent details is recommended however before instituting inquiries or requests.

## Plasma Protein Levels In Normal Individuals

Useful data for plasma protein levels have been published by D. F. Milam (*J. Lab. Clin. Med.* **31,** *285* (*1946*)) on the basis of more than 1500 semi-micro-Kjeldahl determinations of total plasma proteins and albumin in as many different individuals. These data were obtained in a nutrition survey carried out over a period of five and one half years in North Carolina between 1940 and 1945. The entire study pertained to individuals in families who were presumably in ordinary health, living in their customary environment, and at their usual occupation. Their nutritional status was that prevailing in the region studied at the time and they exhibited no signs of protein malnutrition. The mean intake of protein by adults was 60 to 90 g. daily, varying with the year and the area of survey; that of men was usually above 80 g. Oxalated plasma was used for the determinations and consequently the results are not strictly comparable with those of surveys in which serum was employed.

The mean total protein level for white persons was 7.19 g. per cent and for Negroes 7.44 g. per cent. The mean albumin was higher among whites than among Negroes (4.65 and 4.45 g. per cent respectively). The globulin level was therefore higher in the Negro (3.04 versus 2.59 g. per cent). Mean A/G ratios were 1.80 among whites and 1.46 among Negroes. The difference

between the races in globulin levels was twice as great as that between age and sex groups of the same race. The data appear satisfactory and "normal" curves fit the distributions in each racial group very well.

The standard deviation for total proteins was 0.43 for white persons and 0.469 for Negroes. For globulins it was about the same, and slightly lower standard deviations were obtained for albumin. The limits of one half of the distribution of data for total proteins in white persons were 6.84 to 7.42 g. and in Negroes 7.07 to 7.70 g. The corresponding figures for albumin were 4.35 to 4.84 and 4.18 to 4.62 while the figures for globulin were 2.25 to 2.82 and 2.70 to 3.28 respectively.

This is by far the largest group for which data for plasma proteins have been reported. The data correspond well with the average figure ($6.95 \pm 0.02$ g. per cent) of the hitherto largest series, that of J. B. Youmans and co-workers (*Am. J. Pub. Health* **33,** *955 (1943)*) in a survey of 776 individuals in Tennessee.

### Storage of Hendecanoic Acid by the Rat

Hendecanoic acid was chosen as a representative of the "odd carbon" fatty acids of intermediate chain length by F. E. Visscher (*J. Biol. Chem.* **162,** *129 (1946)*) in a study of the disposal of acids of this type by the rat. The lower fatty acids such as butyric and caproic acids are not stored as such, lower odd carbon acids are in part oxidized to ketone bodies, and higher "even carbon" acids are largely laid down in the fat depots. The fate of hendecanoic (or undecylic) acid was followed by feeding a glyceride mixture prepared from hendecanoic acid. One mole of glycerol was esterified with 2 moles of undecylic acid.

Two groups of 18 rats each, of average weight 137 g. were maintained for six weeks on a 25 per cent casein diet. One group received 25 per cent of hendecanoic glycerides in the diet, and the other received 25 per cent of coconut oil; the former group grew 0.9 g. per day and the latter grew 1.2 g. per day.

At the end of the six weeks the rats were anesthetized with sodium pentobarbital, then bled, and the stomach, intestines, spleen, and kidneys were removed. Each carcass was then dissolved in cold 30 per cent KOH over a period of several days. The lipids were obtained for analysis by acidification of the pooled basic solutions and extraction with petroleum ether. The fatty acids were separated, and divided into "solid" and "liquid" methyl ester mixtures which were fractionated by distillation. On the assumption that hendecanoic acid was the only odd carbon fatty acid present, the percentage composition of the fatty acid mixture from the depots was calculated from the analytical data to be 0.3 $C_{10}$, 23.9 $C_{11}$, 2.8 $C_{12}$, 25.4 $C_{16}$, 43.6 $C_{18}$, and 0.5 $C_{20}$ acids respectively. Fat from rats on a low-fat diet contained 0.1 $C_{12}$, 3.1 $C_{14}$, 42.3 $C_{16}$, 53.0 $C_{18}$, and 1.3 $C_{20}$ acids.

The point is therefore established that large quantities of hendecanoic acid can be deposited in the body when this acid is freely supplied in the diet. No evidence was obtained which would indicate that higher odd carbon fatty acids are synthesized from hendecanoic acid, but the methods of analysis were not sufficiently delicate to warrant the definite statement that none at all is synthesized.

### Army Nutrition Laboratory Award

The Surgeon General of the Army has recently awarded the Meritorious Service Unit Plaque to the Medical Nutrition Laboratory for "superior performance of an exceptionally difficult mission and for untiring devotion to duty of the personnel of this unit."

# THE NUTRITION FOUNDATION

The Nutrition Foundation was organized by manufacturers of the food industry to carry out a program of public service.

The purposes of the Foundation are:

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